washed with approximately 1 L of diafiltration buffer. The was is combined with the concentrate to form the Q-load.

An Amicon column (7.0 cm diameter) was packed with approximately 700 ml of Q-Sepharose high performance medium (Pharmacia Q-Sepharose HP). The column was packed with 20% ethanol at 20 psi. The bed height after packing was approximately 18 cm. The column was equilibrated with 5 CF of 6 M urea, 0.02 M Tris/HCl buffer, pH 8. The target for protein loading is 8-10 mg protein/ml Q Sepharose resin. The Q load was applied to the column at a flow rate 30-35 ml/min (50 cm/hr). After loading, the column was washed with approximately 5 CV of 6 M urea, 20 mM Tris/HCl buffer, pH 8.0, or until the absorbance at 280 nm returned to baseline. The product was eluted using a sodium chloride gradient from 0-0.15 M NaCl in 6 M urea, 20 mM Tris/HCl buffer, pH 8.0 over 25 column volumes. The first seven column volumes were collected as a single fraction, followed by 30 fractions of 0.25 column volume each.

Fractions are routinely analyzed by reducing and non-reducing SDS-PAGE and size exclusion chromatography. Fractions are pooled based on aggregate content (<5% by SEC HPLC Method MSL 13929) and qualitative evaluation by SDS PAGE to assess purity. The fractions are stored frozen at -20°C until pooled.

Acceptable Q Sepharose fractions were pooled, and the pH of the pool was adjusted to 7.2 using 2 M HCl. The pool was then concentrated approximately 5 fold in an Amicon DC-1 ultrafiltration system containing a S1Y1 Amicon YM-10 cartridge (10,000 MWCO spiral cartridge membrane). The concentrated Q Pool was then diafiltered against seven column volumes of 2 M urea, 0.15 M NaCl, 20 mM sodium phosphate buffer, pH 7.2. Following ultrafiltration, the solution was drained from the ultrafiltration system. Approximately 100 ml of 2 M urea, 0.15 M NaCl, 20 mM sodium phosphate buffer, pH 7.2 was circulated through the ultrafiltration system for approximately 5 min. The rinse solution was combined with the original concentrate and the solution was filtered through a 0.45 micron vacuum filter unit (Nalgene).